Induction of Highly Embryogenic Calli and Plant Regeneration in Upland (*Gossypium hirsutum* L.) and Pima (*Gossypium barbadense* L.) Cottons

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ABSTRACT

To accomplish our objective of broadening the number of regenerable cotton lines, we developed a protocol capable of producing plants through somatic embryogenesis of diverse cotton species. Callus was initiated from hypocotyl and cotyledon explants on a callus initiation medium [CIM; modified MS with 1 mg L^{-1} kinetin and 2 mg L^{-1} naphthaleneacetic acid (NAA)]. Friable embryogenic callus was periodically selected and transferred onto callus selection/maintenance medium (CS/MM) [modified MS with 0.1 mg L^{-1} kinetin and 0.5 mg L^{-1} NAA]. The selected callus was then transferred into a liquid embryo initiation medium (EIM) (modified MS medium in which NH_4NO_3 was removed and KNO_3 amount doubled) followed by transfer to solid embryo maturation media $EMMS_2$ (0.5 mg L^{-1} NAA + 0.05 mg L^{-1} kinetin). The liquid step not only decreased the culturing time but also increased the number of embryos per gram of cultured tissue. Germinating somatic embryos were placed on MS medium with no hormones and plantlets were acclimatized before transfer to the greenhouse. Significant numbers of somatic embryos and their derived plantlets were obtained from a commercial cultivar of *G. hirsutum*, Deltapine 90 and *G. barbadense* accession GB-35B126 (PI-528306). The mean embryos per gram for Deltapine 90 on $EMMS_2$ were higher than those previously reported for Coker 312. Highly significant differences were found between the two genotypes for both embryo and plant production. To our knowledge, this is the first report of regeneration of *G. barbadense* through somatic embryogenesis.

Gene transfer into plants depends on having well-established tissue culture systems to regenerate plants from a single transformed cell. Whether *Agrobacterium* or biolistics is used, successful transgenic plant production is predicated on generating large numbers of independently transformed plants for screening and evaluation. Somatic embryos offer, among other advantages, the potential for obtaining these numbers. Currently, there are over 7000 cotton accessions at the National Cotton Germplasm Collection at College Station, TX (USDA/ARS, GRIN 2000), and nearly 100 cotton cultivars under cultivation in the USA (Gould et al., 1991; Hemphill et al., 1998), but they are, for the most part, recalcitrant to embryogenesis. In fact, since the first published report of regeneration in *G. hirsutum* (Davidonis and Hamilton, 1983), efforts to regenerate commercial varieties of cotton through embryogenesis have not been particularly fruitful. Most of the successful reports of Upland cotton regeneration involve the Coker lines (Trolinder and Goodin, 1987; Firoozabady and DeBoer, 1993). The easily regenerable Coker lines, though agronomically poor, are the base of the current generation of commercial transgenic cottons, which are obtained by backcrossing a transgenic Coker plant to an elite cultivar. Identification of additional regenerable commercial cultivars of cotton would be highly beneficial to accelerating the development of transgenic Upland cottons.

Pima (*G. barbadense* L.) cottons are becoming popular in the U.S. cotton market because of their superior fiber quality and fineness. There have been some published attempts at regenerating Pima cotton through tissue culture, but all the successful reports that we are currently aware of deal with shoot apex regeneration (Gould et al., 1991). Application of shoot apex regeneration is often limited in transformation research because it sometimes produces undesirable chimeric tissues. Trolinder and Xhixian (1989) screened 38 *Gossypium* genotypes, including a *G. barbadense* accession for which they found a reduced level of embryogenesis compared with Coker 312 (Chen et al., 1987; Trolinder and Goodin, 1988; Trolinder and Xhixian, 1989). However, there was no indication of embryo production or plant regeneration in these reports for the *G. barbadense* accession studied. To our knowledge, there has been no successful regeneration of *G. barbadense* through embryogenesis.

The recalcitrance of cotton to tissue culture has not only slowed the development of transgenic cottons but has also narrowed their genetic base. In addition, most programs utilizing transgenes rely heavily or exclusively on backcrossing to incorporate foreign genes into a desired genotype, a strategy that does nothing to expand genetic diversity (Cotton Crop Germplasm Committee, 1997). U.S. farmers are increasingly depending on transgenic cotton lines to avoid environmental regulation and be cost effective. In addition, the yield in cotton production has reached a plateau stage because of the narrow genetic base. Therefore, there is a pressing need to find new and more regenerable cotton lines. We report here successful regeneration of a commercial Upland cotton, cv. Deltapine 90 and a Pima accession, GB-35B126 (PI-528306).

MATERIALS AND METHODS

Plant Materials

The accessions discussed here are a commercially grown *G. hirsutum* cultivar, Deltapine 90, and a *G. barbadense* accession, GB-35B126 (PI-528306). These plant materials were provided by Dr. A.E. Percival, Curator of the National Cotton Germplasm Collection, USDA/ARS, College Station, TX.

Experimental Design and Statistical Analysis

The experiment was a randomized complete block design, consisting of a factorial arrangement of two cotton accessions, three types of media, two replications, and five plates as sam-

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ples nested within each experimental unit. Statistical analyses were performed by the SAS software (SAS Inst., Cary, NC). Embryos were obtained from both hypocotyl and cotyledon explants.

Seed Sterilization and Germination

Seeds were surface sterilized in consecutive washings of 100% ethanol (30 s) and 23% commercial bleach [5.25% (v/v) NaOCl] (shaken for 20 min at 110 rpm), rinsed three times with and then stored overnight in sterile distilled water. The next day, the seed coats were removed and the seeds placed on a modified MS (Murashige and Skoog, 1962) medium containing 0.49 mg L^{-1} nicotinic acid, 0.82 mg L^{-1} pyridoxine, 1.35 mg L^{-1} thiamine, 20 g L^{-1} glucose, 2 g L^{-1} Gelrite (Merck & Co., Inc., Rahway, NJ) and 0.75 mg L^{-1} $MgCl_2$ (pH 6.8). Plants were germinated under dark conditions for 2 to 3 d and then transferred to a 16 h light, 8 h dark light condition at 28 ± 2°C.

Initiation, Selection, and Maintenance of Embryogenic Callus

Explants used for callus initiation consisted of 4- to 10-d-old hypocotyls and cotyledons. Hypocotyl segments (5-mm sections) were longitudinally split and each cotyledon was cut into 7 to 8 pieces. These hypocotyl and cotyledon sections were aseptically transferred to Petri dishes containing callus induction medium (CIM), which was made up of MS salts supplemented with 0.4 mg L^{-1} thiamine, 2.0 mg L^{-1} NAA, 1.0 mg L^{-1} kinetin, 100 mg L^{-1} myo-inositol, 30 g L^{-1} glucose, 2 g L^{-1} Gelrite, and 0.75 g L^{-1} $MgCl_2$ (pH 5.8). After about 4 wk, preembryogenic callus formed on most plates for both Deltapine 90 and GB-35B126, but this step sometimes required up to 7 wk for some individual plates. Preembryogenic callus was selected based on the morphology and characteristics as described earlier (Firoozabady et al., 1987; Firoozabady and DeBoer, 1993; Rajasekaran et al., 1996; and Sakhanokho et al., 1998). Briefly, this morphology generally included hard green tumorous callus, watery brownish callus, prolifically growing and loose callus with elongated cells, and prolifically growing and loose callus but with smaller cells and very dense cytoplasm. Cotton callus morphology differed with growth regulator combinations (Trolinder and Goodin, 1988; Firoozabady and DeBoer, 1993). Embryogenic cotton calli can also be described as friable cream-color, granular, mid-friable, and yellowish-green. These calli contain globular and heart-shaped proembryoid structures (Firoozabady and DeBoer, 1993).

The prolifically growing and loose preembryogenic callus with smaller cells and very dense cytoplasm was selected and then transferred onto a callus selection/maintenance medium, CS/MM. CS/MM consists of MS salts supplemented with 0.4 mg L^{-1} thiamine, 0.5 mg L^{-1} NAA, 0.1 mg L^{-1} kinetin, 100 mg L^{-1} myo-inositol, 30 g L^{-1} glucose, 2 g L^{-1} Gelrite, and 0.75 g L^{-1} $MgCl_2$ (pH 5.8). After 3 to 4 wk, the preembryogenic callus was further selected and transferred onto fresh CS/MM medium until a healthy growing friable callus was obtained for transfer to liquid culture. This step took from 5 to 8 wk. Callus initiation and maintenance took place at 28 ± 2°C under conditions of 16 h light and 8 h dark, with a light intensity of 70 μmol m^{-2} s^{-1} in all cases.

Liquid Culture Step

Potential embryogenic calli, identified as sections of small, less vacuolate and densely cytoplasmic cells, selected and proliferated on CS/MM medium, were then transferred into liquid somatic embryo initiation medium (EIML). EIML medium consisted of MS salts in which NH_4NO_3 was removed and the amount of KNO_3 was doubled, following the modified media of Davidonis and Hamilton (1983) and Zhang et al. (1991), and supplemented with 10 mg L^{-1} thiamine, 100 mg L^{-1} myo-inositol, 1 mg L^{-1} nicotinic acid, 1 mg L^{-1} pyridoxine, 1 g L^{-1} glutamine, 0.5 g L^{-1} asparagine, and 30 g L^{-1} glucose (pH 5.8). One to 3 g of selected friable embryogenic callus were transferred into 250-mL, wide-mouth flasks containing 100 mL of EIML. These flasks were shaken at 130 rpm under a 16 h/8 h light/dark cycle at 28°C. The cultures were checked on a regular basis to monitor development of somatic embryos.

Embryo Development/Maturation Media

At the end of a 4-wk period, cultures showing embryos or embryoid structures were aseptically strained through a tea strainer and 40-mesh screens to enrich for embryogenic cells that were then placed on various development/maturation media ($EMMS_0$, $EMMS_1$, $EMMS_2$, $EMMS_3$, and $EMMS_4$). Cultures lacking embryos or embryoid structures were transferred into fresh EIML medium and shaken for up to another 4 wk before being processed as above. These development/maturation media, $EMMS_0$, $EMMS_1$, $EMMS_2$, $EMMS_3$, and $EMMS_4$, were similar to EIML but contained, in addition to the Gelrite gelling agent, various NAA or 2,4-D and kinetin combinations. $EMMS_0$ (0 mg L^{-1} NAA + 0 mg L^{-1} kinetin), $EMMS_1$ (1 mg L^{-1} NAA + 0.1 mg L^{-1} kinetin), $EMMS_2$ (0.5 mg L^{-1} NAA + 0.05 mg L^{-1} kinetin), $EMMS_3$ (0.1 mg L^{-1} NAA + 0.01 mg L^{-1} kinetin), and $EMMS_4$ (0.1 mg L^{-1} 2, 4-D + 0.5 mg L^{-1} kinetin) were tested for their ability to develop and mature somatic embryos. This step required 3 to 10 wk and often included the transfer of the plant material to fresh media after 3 to 4 wk. After development, mature somatic embryos were transferred onto various modified MS germination/rooting media containing different levels of IAA: MS0 (MS + 0 mg L^{-1} IAA), MS1IAA (MS + 1 mg L^{-1} IAA), and MS2IAA (MS + 2 mg L^{-1} IAA) for further development. Ungerminated embryos placed on MS2IAA callused and were put back into liquid culture for a second cycle.

Plant Regeneration and Acclimatization

Somatic embryos with two well-developed cotyledons and with or without root systems were transferred to baby food jars containing hormone-free MS medium for further development and acclimatization. Once these embryos or plantlets developed root systems and had reached at least the three-leaf stage, they were transferred to pots containing presoaked Pro-Mix (Premier Horticulture, Inc., Red Hill, PA), which were then covered with plastic bags to allow for gradual acclimatization. After 1 to 2 wk and depending on the vigor of the plants, the plastic bags were gradually opened and eventually removed to allow for their normal growth.

RESULTS AND DISCUSSION

Seed Germination

Poor and nonuniform germination was a problem at the beginning of the experiment because of the variability in water absorption and, possibly, seed age and vigor. We were able to overcome this problem by soaking the sterilized seeds in distilled water overnight and aseptically removing the seed coats before transfer to the germination medium the next day. This technique is relatively easy, and it proved to be useful in improving the germination rate. This improved germination rate may be particularly useful when there is a need to submit a uniform set of seedlings to a treatment. In addition,

this technique helped reduce the contamination problem arising from the seed coats.

Initiation, Selection and Proliferation of Embryogenic Callus

CIM medium, described earlier in the Materials and Methods section, as well as MS2NK medium, which consisted of MS salts supplemented with 2 mg L^{-1} NAA, 0.1 mg L^{-1} kinetin, were originally used for callus initiation for both hypocotyl and cotyledon explants, but the latter medium resulted in excessive root formation in cotyledonary explants. Also, we observed some root formation when lower portions of the hypocotyls were used for callus initiation. Though root formation does not seem to deter embryogenesis (Trolinder and Goodin, 1987), it does reduce the amount of available embryogenic callus. In some cases, excessive root formation with MS2NK resulted in a total lack of callus formation in cotyledons. The CIM medium (Rajasekaran et al., 1996; Sakhanokho et al., 1998) was used for subsequent callus induction for both hypocotyl and cotyledon explants. Hypocotyl callus production was optimized by longitudinally dissecting hypocotyls, allowing more cut surface to be in direct contact with the initiation medium. This medium easily generated callus in all the accessions studied and in both hypocotyl and cotyledon explants. Hypocotyls produced more callus than cotyledons as has been reported by Trolinder and Goodin (1988) and Sakhanokho et al. (1998).

A genotype's capability to produce huge amounts of callus is not indicative of its regenerative capacity. Very often, only a small portion of the callus, formed on both hypocotyl and cotyledon explants, showed embyrogenic potential. Callus selection is an important step in cotton tissue culture, considering the diverse nature of callus morphologies. Several authors have characterized and described embryogenic cotton calli (Firoozabady et al., 1987; and Rajasekaran et al., 1996; Shoemaker et al., 1986). Identifying and selecting potential embryogenic calli is a crucial step in cotton regeneration. Failure to separate embryogenic from nonembryogenic calli often resulted in the potentially embryogenic calli turning nonembryogenic. Not all individual seedlings within an accession gave rise to somatic embryos. Similar observations have been recorded earlier (Trolinder and Xhixian, 1989). Embryogenic callus is generally friable, granular, and yellowish-green. After initiation, the embryogenic callus was selected, proliferated, and transferred three to four times every 2 to 4 wk on CS/MM medium. The embryogenic callus was then transferred to the somatic embryo (SE) initiation medium (liquid medium, EIML). As on solid media, callus morphology and callus color in liquid culture varied drastically, from yellowish-green to black.

The liquid medium not only consistently initiated and produced more SEs than the solid media used for this purpose but it also sped up the process as large numbers of SEs could be produced in only 4 wk (Fig. 1A,B). In this study, only GB-35B126 formed a few sporadic embryos on solid medium. No embryos were formed on solid media alone for Deltapine 90, even after 8 wk, which led to the inclusion of the liquid step in this protocol. Improved performance has been reported in many plant species when cultured on liquid-based medium as compared to solid medium. Examples include *Prunus persica* (L). Batsch (Hammerschlag, 1982), *Triticum aestivum* (L.) em. Thell. (Jones and Petolino, 1988), and in *Gossypium* spp. (Finer, 1988; Gawel and Robacker, 1990). It has been argued that the high medium-to-tissue contact is one of the major factors explaining the greater rate of somatic embryo proliferation in liquid media (Finer, 1988; Gawel and Robacker, 1990).

After transfer on SE development media, more embryos developed on $EMMS_2$ and $EMMS_3$ than on $EMMS_4$ for both accessions (Table 1). Preliminary results obtained with $EMMS_0$ and $EMMS_1$ indicated that these two media performed very poorly (data not shown), so they were dropped from further testing in the study. No substantial difference was noticed between the two species as to the length of time required for the cultures to become embryogenic. Hundreds of tulip-shaped embryos per plate developed on both $EMMS_2$ and $EMMS_3$ (Fig. 1, A–C). However, not all of these SEs developed into normal plants with meristems and roots. In an effort to promote germination and rooting efficiency, SEs were transferred onto MS2IAA, which promoted root development and the germination of few plants, but a large portion of the SEs began to degenerate into callus. This observation led us to decrease the IAA concentration and test MS0 (MS + 0 mg L^{-1} IAA), MS0.01IAA (MS + 0.01 mg L^{-1} IAA), MS1IAA (MS + 1 mg L^{-1} IAA). Better germination efficiency was obtained with MS1IAA.

Embryo Production and Plant Regeneration as Affected by Media

Price and Smith (1979), Trolinder and Goodin (1988), and Zhang et al. (1991) obtained 18, 780, and 190 embryos per gram of callus using an accession of *G. klotzschianum* and two *G. hirsutum* lines, Coker 312, and Coker 201, respectively. The results that we obtained using $EMMS_2$, $EMMS_3$, and $EMMS_4$ were in most cases comparable or better. The mean embryos per gram (914 embryos g^{-1}) obtained with $EMMS_2$ for Deltapine 90 (Table 1) was higher than even those achieved with Coker 312, the variety of choice for cotton embryogenesis. The same medium produced significantly more embryos per gram in GB-35B126 than in either $EMMS_3$ or $EMMS_4$ (Table 1).

The sample mean square was used to test the interaction between replications and treatments, which was found not to be significant; therefore, a pooled error was used to test both the genotype × medium interaction as well as the main factors (Table 2). There was no significant genotype × media interaction, but highly significant differences were found between the two genotypes for both embryos per gram and plants per gram production (Table 2). For media, there was a significant difference in embryo numbers but not in plant production (Table 2), possibly due to competitive effects from overcrowding of SEs as reflected in the percentage of plants obtained from somatic embryos (Table 1).

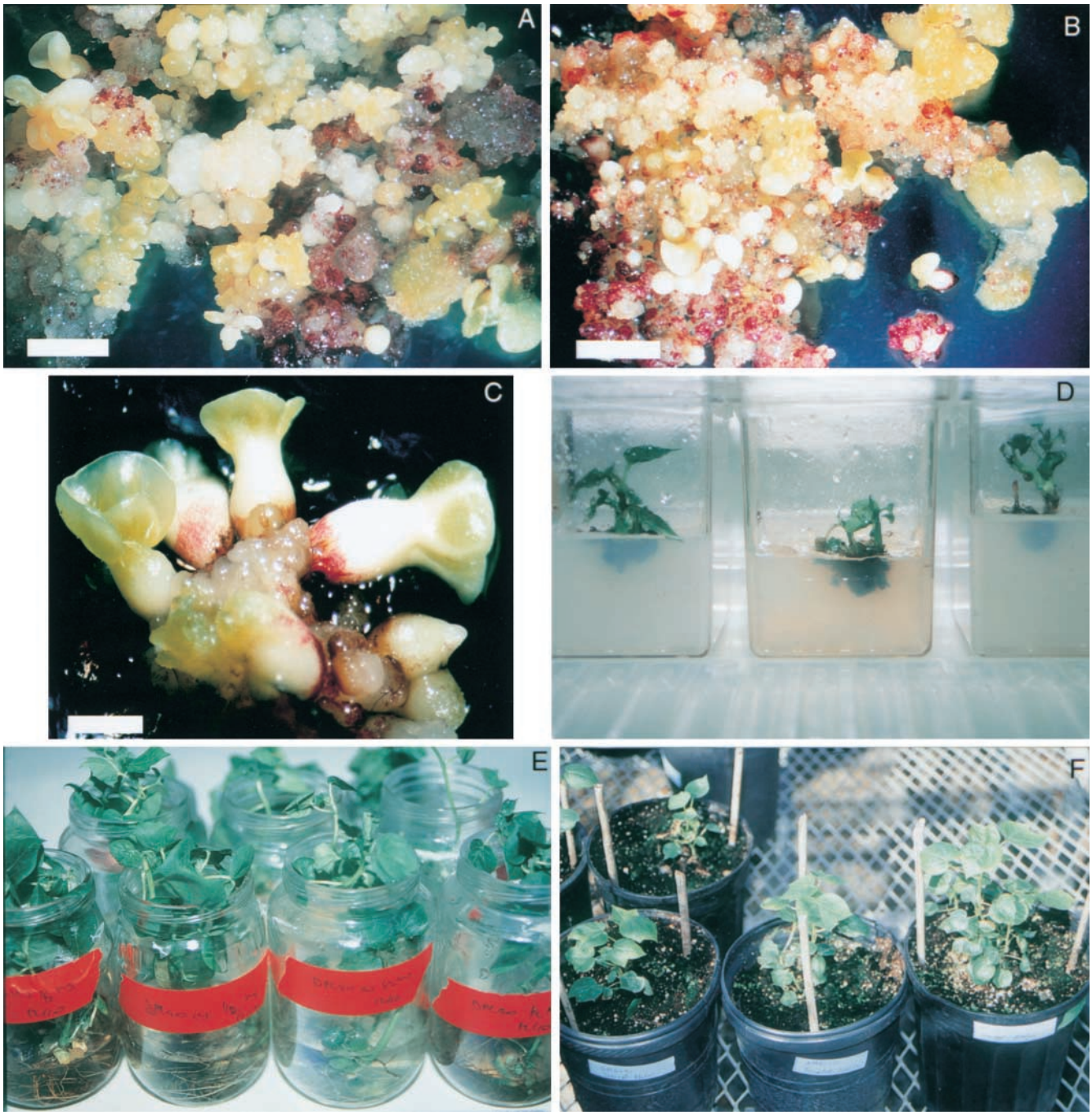

Fig. 1. Regeneration of tetraploid cotton. (A,B) Embryogenic cultures. Note the large numbers of immature and mature somatic embryos. (A) *Gossypium barbadense* accession GB-35B126. (B) *Gossypium hirsutum* cv. DPL90. (C) Mature somatic embryos of *Gossypium hirsutum* cv. DPL90. (D) Rooting plantlets of *Gossypium barbadense* accession GB-35B126. (E) Rooted plantlets of *Gossypium hirsutum* cv. DPL90. (F) Acclimatized plants of *Gossypium hirsutum* cv. DPL90. Scale bar is 10 mm for (A), 7.5 mm for (B), and 3 mm for (C).

Occurrence of Abnormal Embryo and Plantlet Formation

Abnormal embryo and plantlet formation in cotton tissue culture has been reported before (Firoozabady and DeBoer, 1993; Trolinder and Goodin, 1988; Zhang et al., 1991; Zhang et al., 2000). This common occurrence of abnormal embryos and plantlets in cotton tissue culture is most likely due to the length of time generally required by cotton tissue culture. We observed various types of abnormal embryos and plantlets. At times, there were bare stems with no roots or cotyledons. Other embryos produced only roots but no cotyledons. We were not able to regenerate plants from these two types of abnormal embryos. Some authors (Firoozabady and DeBoer, 1993; Zhang et al., 1991) have reported inducing callus from these abnormal embryos and plantlets and subsequently obtaining normal plants, but

Table 1. Mean number of somatic embryos and plants per gram of embryogenic callus and percentage of regenerated plants in two tetraploid cottons cultured on three different media.

Genotype	Medium	Mean embryos g^{-1}†	Mean plants g^{-1}†	%Plants†
Deltapine 90	$EMMS_2$	914.0 ± 9.8	107.4 ± 1.8	11.8 ± 0.4
Deltapine 90	$EMMS_3$	395.5 ± 8.9	92.9 ± 0.6	23.5 ± 0.1
Deltapine 90	$EMMS_4$	346.0 ± 3.5	87.4 ± 0.2	25.3 ± 0.7
GB-35B126	$EMMS_2$	92.2 ± 0.5	24.9 ± 0.2	27.0 ± 0.1
GB-35B126	$EMMS_3$	88.2 ± 1.1	16.3 ± 0.1	18.5 ± 0.2
GB-35B126	$EMMS_4$	38.5 ± 0.2	14.7 ± 0.8	38.2 ± 0.2

† Values represent means ± SD of the number of embryos and plants per gram of embryogenic callus and percentage of plants per gram obtained for each treatment. Each treatment consisted of 20 plates replicated twice for a total of 40 plates.

they presented no somaclonal variation studies showing whether those plants were normal. On the other hand, embryos developing only one cotyledon or possessing callus on the stem could still be induced to produce normal-looking plants. Occurrence of abnormal embryos and plantlets was higher (about 5%) in the *G. barbadense* accession, GB-35B126, than in the *G. hirsutum* cultivar, Deltapine 90 (<1%).

Differential Plant Acclimatization Response

The protocol allowed the recovery of plants, from seed germination to callus culture to soil, in 8 to 9 mo (Fig. 1, D–F). Hardening of recovered plants varied depending on the accessions. Plantlets of the cultivar Deltapine 90 (*G. hirsutum*) were easily hardened in about 7 to 10 d. On the other hand, though similar in vigor to those of Deltapine 90, plantlets of GB-35B126 (*G. barbadense*) in Magenta (Magenta Corp., Chicago, IL) or Mason jars were somewhat difficult to harden and required longer time for acclimatization (3–4 wk). The reason for this phenomenon is unknown.

CONCLUSION

The recalcitrance of commercial cotton varieties to tissue culture has been a major a stumbling block for transgenic cotton development. In addition, the fact that the current generation of transgenic cottons is based on only the Coker lines could lead to a genetic bottleneck problem. The tissue culture protocol developed has generated large amounts of embryogenic callus and copious numbers of somatic embryos and plants in both *G. hirsutum* and *G. barbadense* accessions. The $EMMS_2$ medium, in particular, produced excellent results for both accessions, but particularly for Deltapine 90. We are currently testing the embryogenic potential of some diploid (*G. arboreum* L. and *G. herbaceum* L.) using the protocol described in this paper. Deltapine 90 is an agronomically improved cultivar with good fiber characteristics, so it will have little genetic drift in transgenic backcross programs as compared to Coker 312. Therefore, regenerating plants through embryogenesis in Deltapine 90 constitutes a significant step toward broadening the genetic base of transgenic cottons.

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Table 2. Analysis of variance for the number of embryos and plants per gram produced in two tetraploid cotton accessions.

Source	df	Mean squares: Embryos g^{-1}	Mean squares: Plants g^{-1}
Replication	1	276 255.45ns	763.13ns
Genotype (G)	1	3 666 963.56**	89 527**
Medium (M)	2	504 582.49*	1 248.08ns
G × M	2	404 034.99ns	121.13ns
Error	53	161 774.82	3 042.75

* Indicates significance at $P < 0.05$.
** Indicates significance at $P < 0.01$.
NS, not significant at $P \geq 0.05$.

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Comparison of Molecular Linkage Maps and Agronomic Trait Loci between DH and RIL Populations Derived from the Same Rice Cross

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ABSTRACT

Doubled haploid (DH) and recombinant inbred line (RIL) populations are two types of permanent populations for rice (*Oryza sativa* L.) breeding and genetic mapping. In this study, we report the comparison of molecular maps and mapped agronomic trait loci between DH and RIL populations derived from the same rice cross, ZYQ8 (*indica*) × JXI7 (*japonica*). We investigated six agronomic traits (days to heading, plant height, number of spikelets per panicle, number of grains per panicle, 1000-grain weight, and seed set percentage) and found that five of them did not show significant differences between the two populations. Restriction fragment length polymorphism (RFLP) and microsatellite markers were selected to construct two linkage maps of the DH and RIL populations. All the DNA markers except G39 showed the same linkage groups and orders between the two populations. The genetic distance per chromosome in the RIL population was shorter than that in the DH population, and the total genetic distance of genome in the RIL population (1465 cM) was 70.5% of that in the DH population (2079 cM). In the RIL population, 27.3% markers showed distorted segregation at $P < 0.01$ level, of which 90% markers favored *indica* alleles, while in the DH population, the skewed markers favoring *indica* and *japonica* alleles were in accordance with 1:1 ratio. Eight commonly distorted regions on chromosomes 1, 3, 4, 7, 8, 10, 11, and 12 were detected in both RIL and DH populations, of which seven skewed toward *indica* alleles and one toward *japonica* allele. Five of them were located near gametophytic gene loci (*ga*) and/or sterility gene loci (*S*). We also compared the quantitative trait locus (QTL) mapping results between the DH and RIL populations and found a number of similarities in the QTL locations between these two populations. So both RIL and DH populations are equally effective in rice breeding and genetic analysis.

Since 1988, when McCouch et al. reported the first molecular linkage map of rice, many linkage maps based on various rice populations have been constructed and widely used for mapping QTLs, positional cloning, and comparative genome research (Causse et al., 1994; Song et al., 1995; Gale and Devos, 1998; Harushima et al., 1998; He et al., 1998). Most of maps were independently constructed from either segregating F_2 or backcross populations. These populations are highly heterozygous and cannot be propagated indefinitely through seed. Linkage maps based on DH or RIL populations are permanent and suitable for sustained genetic studies, especially for QTL identification. In rice, the DH populations can be quickly constructed via anther culture, while establishing a RIL population takes many years of continuous self-pollination. Some comparisons between DH and RIL populations have been made in maize, *Zea mays* L. (Mrigneux et al., 1993), wheat, *Triticum aestivum* L. (Hemy et al., 1988), and rice (Courtois et al., 1993; Antonio et al., 1996). But, so far, few reports are available comparing DH and RIL populations from the same rice cross.

We have developed a rice DH population of 150 lines by anther culturing an F_1 hybrid between *indica* 'ZYQ8' and *japonica* 'JXl7'. The genetic linkage map contains more than 440 DNA markers, including RFLP, amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and microsatellite markers (Shen et al., 1998), and has been used to identify many QTLs controlling yield components, anther culturability, and grain quality (Lu et al., 1996; Liu et al., 1997; He et al., 1998, 1999). Recently, we established a RIL population of 107 lines derived from the same ZYQ8/JX17 cross by the single-seed-descent (SSD) procedure. One hundred fifty-four evenly distributed markers in the DH population map were used to construct the linkage map of the RIL population. In this study, we compared the linkage maps and mapped agronomic trait loci between these two permanent rice populations.

MATERIAL AND METHODS

Experimental Populations and Phenotypic Evaluations

The RIL population was derived from a cross between *indica* 'ZYQ8 and *japonica* JX17 by SSD. One panicle was harvested from each individual ZYQ8/JX17 F_2 plant and one seed from each panicle was chosen randomly and planted in the field to obtain F_3 plants. This procedure was continued in the following generations until F_8 plants were obtained.

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Abbreviations: AFLP, amplified fragment length polymorphism; cM, centimorgan; DH, doubled haploid; LOD, logarithm of odds ratio; PCR, polymerase chain reaction; QTL, quantitative trait locus; RAPD, random amplified polymorphic DNA; RFLP, restriction fragment length polymorphism; RIL, recombinant inbred line; SSD, single seed descent.